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Związek pomiędzy polimorfizmem genu ACTN3 i siłą mięśni u osób starszych

Association between ACTN3 gene polymorphism and muscle strength in older adults

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Abstract

The decrease of muscle strength and general physical fitness is a big problem in the elderly group. It often prevents the proper functioning of a person, which makes him/her dependent on caretakers. It is believed that the some polymorphisms in ACTN3 geneare associated with better results in strength sports. If those polymorphisms affect muscle strength, they may also be related to the health condition of older people. Our study was conducted on a group of 60 people over 60 years of age. The relationship between overall fitness and muscular strength of elderly patients with ACTN R577X gene polymorphism was checked. The ACTN3 gene was tested by PCR-RFLP. Overall fitness was tested using the IPAQ questionnaire and fitness tests: Tinetti, DGI, Up&Go, SPPB, standing on one leg (both right and left). Based on the results of the survey, the MET and BMI coefficients were determined. It was found that the RX genotype is associated with better results achieved in the SPPB test. Genotype XX was associated with worse results in the right leg standing test ($p<0.05$). The R-allele of the ACTN3 gene wasassociated with better results obtained during the standing test on the right leg ($p<0.05$).Based on obtained results it was impossible to determine a clear impact of the ACTN3 gene on muscle strength and health of the older adults.

Key words: sport genetics, ACTN3, older adults, geriatric patient

Streszczenie

Spadek siły mięśniowej oraz ogólnej sprawności fizycznej jest dużym problemem w grupie osób starszych. Często uniemożliwia prawidłowe funkcjonowanie danej osoby, przez co staje się ona zależna od swoich opiekunów. Uważa się, że gen ACTN3 ma związek z lepszymi wynikami osiąganymi w sportach siłowych. Ze względu na swój domniemany związek z siłą mięśni może mieć on również wpływ na stan zdrowia osób starszych. Niniejsze badanie przeprowadzono na grupie 60 osób powyżej 60 roku życia. Poddano analizie związek pomiędzy ogólną sprawnością fizyczną i siłą mięśni starszych pacjentów a polimorfizmem genu ACTN3 R577X. Polimorfizm genu ACTN3 został zbadany metodą PCR-RFLP. Ogólną sprawność badano przy użyciu kwestionariusza IPAQ oraz testów sprawnościowych: testu Tinetti, DGI, Up&Go, SPPB, stania na

jednej nodze (zarówno prawej jak i lewej). Na podstawie wyników ankiety określano współczynnik MET oraz BMI. Wykazano, że genotyp RX ma związek z osiąganiem lepszych rezultatów podczas testu SPPB ($p<0,05$). Genotyp XX powiązany jest z gorszymi wynikami podczas testu stania na prawej nodze ($p<0,05$). Allel R genu ACTN3 jest związany z lepszymi wynikami osiąganymi podczas testu stania na prawej nodze ($p<0,05$). Dodatkowo, występuje on częściej u mężczyzn ($p<0,05$). Bazując na otrzymanych rezultatach niemożliwe jest jednoznaczne stwierdzenie wpływu genu ACTN3 na siłę mięśni i stan zdrowia osób starszych.

Słowa kluczowe: genetyka sportowa, ACTN3, osoby starsze, pacjent geriatryczny

Introduction

Sports genetics is a relatively new branch of genetic research. It focuses mainly on identifying polymorphisms associated with muscle strength or endurance in athletes. Due to the fact that older people often suffer from muscle weakness or muscle loss (sarcopenia), they are an interesting group to carry out such tests.[1] The International Physical Activity Questionnaire (IPAQ) is a tool used to assess the physical fitness of elderly people. It is a questionnaire consisting of a set of questions and physical efficiency tests. The questionnaire contains questions about everyday activities, walks and sports. Italso contains an interview about the state of health, injuries and falls and their consequences. Based on the questionnaire, the Metabolic Equivalent of Work (MET) can becalculated. Determining MET allows to classify a person in terms of physical activity.[2] In the physical efficiency test section, the subject is tested to assess the risk of falling and overall physical condition. The following fitness tests are carried out: Tinetti test, Up&Go, Dynamic Gate Index (DGI test), Short Physical Performance Battery (SPPB test) and one leg standing test. [3–7].Each test has a specific scoring scale and criteria on the basis of which the obtained result is interpreter.[2]

ACTN3 is the gene coding α-actinin type 3. coding gene. It is located on the long arm of 11^{th} chromosome (locus:11q13.2). Actinins are proteins produced in skeletal muscle. The most popular types are α-actinin type 2 and 3. Actinins stabilize the muscle contractile apparatus. The expression of α-actinin type 3, in contrast to α-actinin type 2, which is expressed in all skeletal muscles, is limited only to fast-twitch muscles. Its presence increases the dynamics of contraction and its strength, while reducing muscle endurance for long-lasting effort.[8] The R577X polymorphism alters position 577 of the alpha-actinin-3 protein. It results in a partial deletionof α-actinin 3 protein, which leads to a disruption of the muscle fiber structure and a decrease in muscle strength.[9] Research carried out so far on a group of athletes suggest that the allele R of the ACTN3 gene is associated with better results in strength sports and allele X is associated with better results in endurance sports.[10,11] Identifying the relationship between individual gene variants and ailments of elderly patients can help those patients by using the right set of exercises or medications before the discomfort occurs.

Materials and methods

Subjects

Ourstudy was carried out on a group of people over 60 years of age. To ensure safety during physical tests, this study was carried out in cooperation with physiotherapists. The test consisted of the questionnaire part and the fitness part: one leg standing test, Tinetti test, *Up&Go Test*, Dynamic Gait Index (DGI), Short Physical Performance Battery (SPPB test).. The tests were carried out with the consent of the bioethical commission.

Genetic tests

DNA isolation was performed using a DNA isolation kit from biological traces GeneMATRIXBio-Trace DNA Purification Kit (version 3.1, cat. nr E3510EURx). The ACTN3 gene polymorphism was tested using PCR-RFLP method. The PCR reaction was carried out according to Eduardo MendonçaPimenta protocol. [12] The primers used for the reaction had the following sequence: F - 5'-CTG TTG CCT GTG GTA AGT GGG-3', R -5'-TGG TCA CAG TAT GCA GGA GGG-3' (synthesized by Sigma-Aldrich). The reaction was carried out in a volume of 25 µl.Final concentration of reagents: Green GoTaq Flexi Buffer (Promega) 1x, R and F starters 1 µM, dNTPs 0.2mM (Promega), $MgCl_2$ 1.75mM, GoTaq Hot Start polymerase (Promega) 1U/reaction and about 100 ng template DNA. The PCR reaction was performed in a GeneAmp 2720 Applied Biosystems thermocycler. The amplification program consisted of the following steps: preliminary denaturation at 94°C for 5 minutes, 30 cycles of: denaturation (94°C, 1 minute), annealing (64°C, 1 minute) and elongation (72°C, 1 minute) and with a 5 minute elongation at 72°C. The product of the PCR reaction was digested with restriction enzyme HpyF3I (Thermo Scientific). This process was carried out at 37°C for 16 hours, followed by enzyme inactivation - 20 minutes at 65°C. The digested product was separated on a 3% agarose gel in TAE buffer (Sigma Life Science).

Statistical analysis

The effect of ACTN3 gene variants on individual test results has been checked. The tests that were selected for further analysis were: SPPB, Tinetti, Up & Go, DGI, one leg standing and parameters: BMI, total MET, sex and number of falls. The frequency of

genotypes and alleles for each trait and group was calculated. On this basis, statistical significance was examined using the Statistica 13.1 program. For each feature and its genotypic variant, a two-sided difference was calculated between the two structure indices. If the p-value was less than 0.05 ($p<0,05$), the result was considered statistically significant.[13]

Results

Of all the results, 2 were found to be statistically significant. The difference in the results of the SPPB test is significant - higher scores are achieved by people with the RX genotype. In the one leg standing test on right leg, better results are achieved by people with RX genotypes, while worse people with the XX genotype. Summary of the results of selected tests are shown in Table I.

Table I Summary of the results of selected tests that were carried out. Average values are displayed with standard deviations.The bold scores are showing statistically important results.

Trait/test	ACTN3		
	RR	RX	XX
Numer of people	12	15	7
Age	73,5 (±8,2)	73,6 (±4,6)	75 (±6,8)
BMI	29,33 (±4,96)	30,09 (±5,98)	31,27 (±2,88)
SPPB	11,75 (±3,67)	**14 (±2,03)**	11,71 (±2,62)
DGI	20,92 (±3,53)	20,79 (±3,47)	20,57 (±4,08)
Tinetti	25,67 (±2,42)	26,2 (±2,48)	26,43 (±1,9)
Right leg [s]	11,6 (±8,09)	**11,3 (±7,15)**	5,1 (±5,12)
Left leg [s]	12,72 (±12,58)	12,22 (±14,88)	4,84 (±2,77)
Up&Go [s]	10,95 (±4,98)	9,83 (±3,61)	10,74 (±4,16)
MET	3345 (±2213)	4755 (±5941)	5538 (±3425)

Discussion

Currently researchers are trying to understand the interactions between genes and environment. A popular subject of research are gene relationships with sports results carried out on professional groups of athletes. One of the more frequently studied genes is ACTN3. As the published results are contradictory, thequestion of actual influence of ACTN3 polymorphismson the results achieved in strength sports andphysical fitness of elderly people remains open. In studies carried out by Wang et al., the association of the ACTN3 gene with the results achieved in strength sports was examined. In this case, no relationship was identified. The lack of association was also confirmed in several other studies.[14,15] Research conducted by Papadimitriou et al. on a group of Greek sprinters suggest a close relationship between the RR genotype and better performance in strength sports.[16] The relationship between the genotype RR and better performance in short-distance swimming in women has been demonstrated by Chiu et al.[17] There was no relationship between BMI and genotypic variants of the ACTN3 gene. In the J. Ho Kim study, the same results were achieved.[18]

Our study showed a statistically significant relationship between the RX genotype of the ACTN3 gene and the results of the SPPB test ($p < 0.05$). The RX genotype was more common in people qualified for the group with no physical limitations (60%) than in the group with such limitations (21%). These results are not confirmed by the study conducted by Delmonico. After examining 1367 people aged 70-79, there were no statistically significant differences in the occurrence of genotypes in groups with different efficiencies.[19]As our study was conducted on a relatively small group of patientsand our results has not been confirmed in the literature, there is a possibility that our result is an artifact of small sample size and thus additional analyses on larger group of patients are necessary. The analysis of the MET results suggests that ACTN3 gene polymorphisms are not related. None of the results was statistically significant. Delmonico et al. identified a relationship between the RX genotype and lower physical activity in men ($p<0.05$). In the case of women, there were no differences between the activities in different groups.[19]In our study there was no association of ACTN3 genotypes with the results oftheUp&Go test. In studies carried out by Pereira et al. on a group of women over 57 years of age, no relationship between genotypes was found too ($p>0.05$).[20] The analysis of the relationship between results for right leg in one leg

standing test and the genotypic variants suggests that the XX variant of the ACTN3 gene is associated with worse results achieved in this test ($p<0.05$). The RX genotype of the ACTN3 gene is on the verge of statistical significance ($p=0.0548$) and is potentially associated with better results achieved in this test. In the case of a standing on the left leg test, no statistically significant results were noted. The relationship between the genotype and the results of the equilibrium tests was also examined (Tinetti, DGI). In this case, none of the features was statistically significant.

The obtained results suggest that the association of ACTN3 gene polymorphisms with the efficiency of older people is possible. Further research is needed to demonstrate this relationship.

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